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Study on the binding of farrerol to human serum albumin

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Abstract

Binding of farrerol to human serum albumin (HSA) was investigated at 293, 303, and 313 K and pH 7.40 using spectrophotometric technique and molecular modeling. The binding parameters obtained from the modified Scatchard's procedure were in close agreement with those from Stern–Volmer equation. Based on the thermodynamic parameters calculated from the van't Hoff equation, the enthalpy change ΔH° and entropy change ΔS° for the process of farrerol binding to HSA were evaluated at -18.51 kJ mol^{-1} and 47.52 J mol^{-1} K^{-1}, respectively. The value of 2.63 nm for the distance r between the donor (HSA) and acceptor (farrerol) was derived from the fluorescence resonance energy transfer. From these results, three issues on the interactions between farrerol and HSA could be approached: (a) farrerol is strongly bound to HSA, (b) the primary binding site is located at the site I of HSA, and (c) apart from hydrophobic interactions, there still exist hydrogen bond interactions between farrerol and the residues of HSA, such as Lys195, Arg218, Arg222, and Ala291. Besides, the conformational change of HSA was observed, being caused by the interaction with farrerol.

Keywords: Farrerol; Human serum albumin; Secondary structure; Spectrophotometric; Molecular modeling

1. Introduction

One of the important topics in pharmaceutical research is to learn the interaction of drug with proteins. Drugs can be incorporated into proteins which act as carriers. Among them human serum albumin (HSA) has been considered as a typical representative. HSA consists in blood plasma as a major protein component. Besides, it exists in interstitial fluids. HSA contributes to colloidal osmotic blood pressure and, most importantly, plays a key role in the transport of a wide variety of substances [1,2]. The X-ray crystallographic data for HSA reveal it actually to be a 585 amino acid residue monomer containing three homologous α-helical domains I, II, and III [3]. Each domain is constituted by two sub-domains A and B, which share common structural elements. In crystal these six sub-domains assemble to form a heart-shaped molecule [3]. Both of their aromatic and heterocyclic ligands are bound within two hydrophobic pockets in sub-domains IIA and IIIA, which are referred to as the sites I and II, respectively [2,4–5]. Seven binding sites are localized by fatty acids in sub-domains IB, IIIA, IIIB, and on the sub-domain interfaces [6]. HSA has also a high affinity-binding site to metal at the N-terminus [7]. The multiple binding sites underlie the exceptional ability of HSA to interact with many organic and inorganic molecules. Consequently, it becomes an important regulator of intercellular fluxes and displays a pharmacokinetic behavior in many drugs [8].

Otherwise, most of the flavonoid drugs show a high degree of binding to HSA, which is a primary determinant of their pharmacokinetic properties. The previous reports on these [8–11] involve quercetin, kaempferol, delphinidin, scutellarin, alpinetin, formononetin, etc. Farrerol (Scheme 1) is a new kind of 2,3-dihydro-flavonoid drug and has been synthesized as an antibechic. Investigations have shown that farrerol has a wide spectrum of physiological activities such as anti-inflammatory, anti-bacterial, and antioxidant activity for scavenging radicals and inhibiting a variety of enzymes [12]. Pathological experiments have demonstrated that farrerol relieves coughs and moves phlegm. It has been found that more than 95% of farrerols are primarily bound to HSA, which serves as a storehouse for farrerols, and the unbound moiety is pharmacologically active. Thus, the nature and magnitude of the interactions between farrerol and HSA have important pharmacokinetic and pharmacodynamic implications. Yet, few works have been published for

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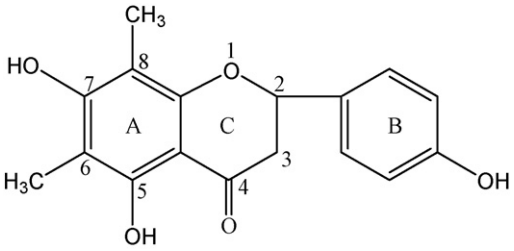

Scheme 1. The structure of farrerol.

the mechanism of the interactions and detailed physicochemical characterizations of farrerol binding to HSA.

Spectrophotometric techniques and molecular modeling studies have been widely used for monitoring drug binding to plasma albumin because of its sensitivity, accuracy, rapidity, and ease of use [13]. In the present paper, studies on the mechanism, mode, and conformation change of farrerol binding to HSA have been performed using spectroscopy and molecular modeling. The results have been discussed on the binding parameters, the identification of binding sites, the effect of farrerol on the conformation of HSA, and the nature of forces involved in the interactions.

2. Materials and methods

2.1. Materials

HSA fraction V was obtained from Sigma and essentially fatty acid free. Farrerol of analytical grade was obtained from National Institute for the Control of Pharmaceutical and Biological Products, China. All other reagents were of analytical grade. Double-distilled water was used throughout experiments. Solutions were prepared in 20 mM phosphate buffered saline (PBS) at pH 7.40, whose ion strengths were kept at 0.1 M. A 35 μM solution of HSA was prepared in a pH 7.40 PBS. A 0.3 mM methanol solution of farrerol was used for all the binding experiments. The concentration of HSA was determined spectrophotometrically on a Shimadzu UV-260 UV–vis spectrophotometer using ε_{280} (HSA) = 36,500 M^{-1} cm^{-1} [8].

2.2. Absorption spectra

UV–vis absorption was measured on a Shimadzu UV-260 UV–vis spectrophotometer in a 1 cm cuvette. The UV–vis spectra were scanned in the range of 240–300 nm and 300–500 nm at room temperature.

2.3. Fluorescence spectra

Fluorescence spectra were recorded on a PE LS55 fluorimeter in a 1 cm quartz cell following an excitation wavelength of 295 nm at a definite temperature over a wavelength range of 300–500 nm. The selection of 295 nm for excitation was to ensure the light absorbed by the tryptophan residue alone. The excitation and emission slits were 10 nm. Quantitative analysis of the interaction between HSA and farrerol was performed with a fluorimetric titration experiment as follows: 3 mL of a 3.5 μM solution of HSA was titrated by successive addition of farrerol solution to reach a final concentration of 6 μM farrerol. Spectrophotometric analysis of the fluorescence quenching was carried out with the Stern–Volmer equation (Eq. (1)) [14]:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = 1 + k_q\tau_0[Q] \quad (1)$$

where F_0 and F are the relative fluorescence intensities in the absence and presence of quencher, respectively, $[Q]$ the concentration of quencher, K_{SV} the Stern–Volmer quenching constant, k_q the quenching rate constant for a biomolecular reaction, and τ_0 is the average lifetime for fluorophore in the absence of quencher evaluated at 10^{-8} s [15]. Linear plots of F_0/F against $[Q]$ yield K_{SV} as slopes, and k_q can be calculated.

The binding parameters for the farrerol–HSA system have been derived from fluorescence quenching data inspected at 293, 303, and 313 K, respectively, according to the modified Scatchard's procedure (Eq. (2)) [16]:

$$\frac{F_0}{F} = K_A\left(\frac{[Q]F_0}{F_0 - F}\right) - nK_A[P] \quad (2)$$

where $[P]$ is the molar concentration of total HSA, $[Q]$ the molar concentration of total farrerol, n the binding stoichiometry per class of binding sites, and K_A the equilibrium binding constant.

The values for the enthalpy change (ΔH°) and entropy change (ΔS°) will be evaluated from the van't Hoff equation [17] by considering ΔH° not varying significantly over the experimental temperature range:

$$\ln K_A = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (3)$$

where K_A is the binding constant at a definite temperature and R the gas constant. A linear plot of $\ln K_A$ against $1/T$ yields ΔH° and ΔS° for the binding interaction. Consequently, the amount of free energy change required for the binding is estimated from Eq. (4):

$$\Delta G^\circ = \Delta H^\circ - T\,\Delta S^\circ \quad (4)$$

2.4. Estimation of binding distance r

Eq. (5) defines E for the energy transfer efficiency based on the Förster's theory, where r is the distance from a ligand to a tryptophan residue of a protein, and R_0 the Förster critical distance, at which 50% of the excitation energy is transferred to an acceptor [18]. The value for r can be calculated from donor emission and acceptor absorption spectra according to the Förster formula (Eqs. (5)–(7)):

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \quad (5)$$

$$R_0^6 = 8.8 \times 10^{-25} K^2 N^4 \Phi J \quad (6)$$

$$J = \frac{\int_0^\infty F(\lambda)\varepsilon(\lambda)\lambda^4\,d\lambda}{\int_0^\infty F(\lambda)\,d\lambda} \quad (7)$$